

The Expression and Localization of Kappa Myeloma Antigen on Malignant and Normal B cells

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requirements for the degree of
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Certificate of Authorship/Originality

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A handwritten signature in dark ink, appearing to read 'A. Hutchinson', with a stylized, cursive script.

Andrew Tasman Hutchinson

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Abbreviations

| | |
|----------------|--|
| - | negative |
| + | positive |
| °C | degrees Celsius |
| α | alpha |
| δ | delta |
| ϵ | epsilon |
| γ | gamma |
| κ | kappa |
| λ | lambda |
| μ | mu |
| μg | microgram |
| μL | microlitre |
| \AA | angstrom |
| Ab | antibody |
| ADCC | antibody dependent cellular cytotoxicity |
| AIDS | acquired immune deficiency syndrome |
| AP | alkaline phosphatase |
| APC | allophycocyanin |
| APCs | antigen presenting cells |
| ATP | adenosine triphosphate |
| Auto-Ab | auto-antibody |
| Az | azide |
| BCR | B cell receptor |
| BiP | immunoglobulin heavy chain-binding protein |
| BJP | bence jones protein |
| b κ Fab | biotinylated κ Fab |
| BSA | bovine serum albumin |
| bVOR | biotinylated VOR |

| | |
|--------------------|---|
| C3 | complement component 3 |
| CCDA | co-stimulatory cell dependent activation |
| CCIA | co-stimulatory cell independent activation |
| CD | cluster of differentiation |
| CDC | complement-dependent cytotoxicity |
| C-domain | constant domain |
| CDR | complementarity determining regions |
| D | diversity |
| Da | dalton |
| ddH ₂ O | double distilled water |
| D _H | hydrodynamic diameter |
| DLS | dynamic light scattering |
| DNA | deoxyribonucleic acid |
| DOPC | 1,2-dioleoyl-sn-glycero-3- phosphatidylcholine |
| DTT | dithiothreitol |
| ECL | enzymatic chemiluminescence |
| ELISA | enzyme-linked immunoabsorbent assay |
| ER | endoplasmic reticulum |
| EWB | enzyme-linked immunoabsorbent assay wash buffer |
| EWB-T | enzyme-linked immunoabsorbent assay wash buffer with tween 20 |
| Fab | fragment, antigen binding |
| Fc | crystallisable fragment |
| FITC | fluorescein |
| FLC | free immunoglobulin light chain |
| FκLC | free immunoglobulin kappa light chain |
| FλLC | free immunoglobulin lambda light chain |
| FSC | forward scatter |
| FSW | flow cytometry staining wash |
| g | gram or centrifugal force |
| GC | germinal centre |
| GFP | green fluorescent protein |

| | |
|--------------|--|
| gp41 | glycoprotein 41 |
| GPI | glycosylphosphatidylinositol |
| GRP | glucose-regulated protein |
| h | hours |
| HC | heavy chain |
| HCD | heavy chain disease |
| HRP | horse radish peroxidase |
| hsp | heat shock protein |
| Ig | immunoglobulin |
| IgA | immunoglobulin A |
| IgD | immunoglobulin D |
| IgE | immunoglobulin E |
| IgG | immunoglobulin G |
| IgM | immunoglobulin M |
| IL | interleukin |
| J | joining |
| k | kilo |
| kDa | kilodalton |
| KMA | kappa myeloma antigen |
| L | litre |
| LC | light chain |
| LCA | light chain amyloidosis |
| LCDD | light chain amorphous deposition disease |
| LMA | lambda myeloma antigen |
| LUV | large unilamellar vesicle |
| M | molar |
| mAb | monoclonal antibody |
| m β CD | methyl β cyclodextrin |
| mg | milligram |
| MGUS | monoclonal gammopathy of undetermined significance |
| MHC II | major histocompatibility complex class II |

| | |
|-----------|--|
| min | minutes |
| mL | millilitre |
| MM | multiple myeloma |
| mM | millimolar |
| M-protein | monoclonal protein |
| mRNA | messenger ribonucleic acid |
| MS | multiple sclerosis |
| MZ | marginal zone |
| nm | nanometer |
| N-Smase | neutral sphingomyelinase |
| OD | optical density |
| p | pico |
| PAGE | polyacrylamide gel electrophoresis |
| PAMPs | pathogen-associated molecular patterns |
| PBMCs | peripheral blood mononuclear cells |
| PBS | phosphate buffered saline |
| PDI | protein disulfide isomerase |
| PE | phycoerythrin |
| PerCP | peridinin-chlorophyll-protein complex |
| PLC | phospholipase C |
| POPC | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine |
| POPE | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine |
| POPS | 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphatidylserine |
| qPCR | quantitative polymerase chain reaction |
| R10 | RPMI 1640 media and 10% foetal bovine serum |
| RA | rheumatoid arthritis |
| RMSD | root mean square deviation |
| RNA | ribonucleic acid |
| RT | room temperature |
| RT-qPCR | reverse transcriptase quantitative polymerase chain reaction |
| RU | arbitrary response unit |

| | |
|----------|---|
| s | second |
| SAC | <i>Staphylococcus aureus</i> cowan I strain |
| SDS-PAGE | sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| siRNA | small inhibitory ribonucleic acid |
| SLE | systemic lupus erythematosus |
| SP-A | surfactant protein-A |
| SPR | surface plasmon resonance |
| SSC | side scatter |
| TLR | toll-like receptor |
| TMNCs | tonsil derived mononuclear cells |
| TX100 | triton X 100 |
| TX114 | triton X 114 |
| V | variable |
| V-domain | variable domain |
| V(D)J | variable, (diversity), joining |
| VH | variable heavy chain |
| VL | variable light chain |
| WM | Waldenström's macroglobulinaemia |
| XBP-1 | x-box binding protein 1 |

Abstract

Kappa Myeloma Antigen (KMA) is a plasma membrane associated form of free immunoglobulin kappa light chain (FκLC) expressed on malignant B cells from patients with multiple myeloma (MM), Waldenström's macroglobulinaemia (WM) and non-Hodgkin's lymphoma (Walker et al. 1985). KMA is recognized by the murine monoclonal antibody (mAb) mKap, and its human-mouse chimeric equivalent, cKap, which is currently undergoing clinical trials as a therapy for kappa type MM (Boux et al. 1983; Raison et al. 2005).

Earlier expression studies on KMA suggested that the antigen is not expressed by normal B cells *in vivo*. However, *in vitro* activation of tonsillar B cells induced expression of KMA on a subset of cells. Like their KMA expressing malignant counterparts, these were presumed to be FκLC secreting plasma cells or plasmablasts but, due to the lack of B cell lineage specific markers at the time, these cells were not phenotyped (Walker et al. 1985). Furthermore, given the extremely low frequency of plasmablasts and plasma cells in normal tissues, it was not possible to exclude the presence of a 'normal' KMA positive cell population *in vivo*.

The first section of this thesis expands upon this earlier work. By utilizing *in vitro* activation protocols on peripheral blood CD19+ B cells, KMA expression was induced on a subset of cells. Phenotypic analysis revealed that the majority of KMA positive cells were CD27++ CD38+/- plasmablasts and CD38++ plasma cells. Analysis from normal human tissues found that a subset of plasma cells in the tonsils expressed the antigen. These cells co-expressed CD45, indicating that they are at an immature stage of plasma cell differentiation. In contrast, peripheral plasma cells, considered to be more fully mature cells in transit from secondary lymphoid organs to plasma cell niches in bone marrow or spleen, did not express KMA. This implies that KMA expression, *in vivo*, is limited to a small subset of immature plasma cells in secondary lymphoid organs such as the tonsils.

Despite cKap's current assessment in clinical trials for the treatment of MM, very little is known about its molecular target KMA. Previous studies have showed that KMA is comprised of FκLC (Goodnow and Raison 1985); however it was never determined as to how FκLC is associated with the plasma membrane. Since FκLC is a secreted molecule, it was initially presumed that it associated with a proteinaceous 'membrane receptor' (Goodnow and Raison 1985). However membrane extraction studies, as described in the second part of this thesis, reveal that FκLC directly associates with the plasma membrane through a combination of hydrophobic and electrostatic forces to form KMA. Further investigations confirmed that FκLCs can bind directly to cellular and artificial membranes. Moreover, this binding is likely dependent on self-association processes, which suggest that KMA consists of aggregated, membrane associated FκLCs.

Lipid binding studies revealed that FκLCs associate specifically with saturated phosphocholine species such as sphingomyelin in membranes, and KMA expression was positively correlated with sphingomyelin expression in FκLC secreting cell lines.

The final section of this thesis examines how FκLCs might interact with saturated phosphocholine lipids. Molecular modeling of dimeric FκLC suggests they are able to weakly associate with phosphocholine in the conventional antigen binding pocket formed by the κLC variable domain (V-domain). Since FκLC aggregation is a feature of KMA, then the avidity effects of multi-valent binding likely increases the strength of the proposed FκLC-phosphocholine interaction. This hypothesis explains the observation of both electrostatic and hydrophobic interactions by FκLC, as KMA, with the plasma membrane - the electrostatic component, governed by single FκLC molecules interacting with the charged phosphocholine headgroups, and the hydrophobic component, due to self-association of adjacent FκLC molecules.

Finally, a model of KMA expression by FκLC secreting cells is proposed. FκLC is synthesized in the endoplasmic reticulum (ER) then transported to the golgi-apparatus and encapsulated into vesicles destined for secretion. There FκLCs interact with saturated

phosphocholine lipids, such as sphingomyelin, and undergo aggregation resulting in stable association on the inner vesicular membrane. Fusion of the vesicle with the plasma membrane during exocytosis allows for membrane associated F κ LC to become exposed on the extracellular face as KMA.